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NUTRITION REVIEWS

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NUTRITION REVIEWS

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ORGANIZATION AND FUNCTIONS OF THE NUTRITION CLINICS OF THE DEPARTMENT OF HEALTH OF THE CITY OF NEW YORK

The organization and functions of the nutrition clinics of the Bureau of Nutrition of the Department of Health of the City of New York are an integral part of its community health services. New York City, with a population of over 8 million people of varied social, economic, ethnic, and religious backgrounds, presents a challenging problem in the administration of its nutrition program as well as all of its other public health programs. Many sections of the city have health needs that rival those of entire communities not only in magnitude but in complexity. The functions of the Department of Health are delegated to eighteen bureaus, grouped into five major services: community health services in which the Bureau of Nutrition is placed, administrative services, preventable diseases and adult hygiene, maternal and child health services, and environmental sanitation.

To cope with the large variety of problems arising in a city like New York, the central office organization of the Bureau of Nutrition operates, like other bureaus, through district health centers in order that its services can be adapted to the needs of the local population. There are thirty district health centers each with populations ranging from 130,000 to 438,000, the average being about 250,000.

The Bureau of Nutrition attempts to improve the health of the people of the City of New York through better nutrition. It approaches this task through four programs:

(1) *Nutrition Education of the Public.* Its program of education directed primarily to the public is in cooperation with the Bureau of Public Health Education through which material for publication, radio talks, and exhibits are presented. It assists Department of Health and public school personnel with school nutrition projects and participates in in-service training courses for teachers. The Bureau of Nutrition also cooperates with other agencies in planning and developing community nutrition programs.

(2) *Education of Patients Attending Department of Health Clinics.* This phase of the educational program is designed for patients attending other Health Department clinics such as tuberculosis, social hygiene, eye, maternal, baby health, and prenatal. Printed materials and teaching aids for the patients are prepared together with the Bureau of Public Health Education and the bureaus responsible for the clinics.

(3) *Technical Advisory Services.* The Bureau also offers a technical advisory service for clinicians and nurses concerned with the dietary problems of patients attending the Department clinics, and for directors of nursery schools and other agencies giving group care to children. The service prepares reports of recent research on special topics, cooperates with public and private health and welfare agencies in determining adequate food allowances of minimum cost for various age groups, and participates in periodic pricing of these allowances. It also supervises field work in public health nutrition for graduate students and takes part in courses on nutrition for graduates and undergraduate students of medicine, nursing, and home economics.

(4) *Direct Services Through Its Nutrition Clinics.* The purposes of the Bureau's nutrition clinics are: to furnish consultation, diagnostic, and follow-up service for people suspected of having nutritional abnormalities; to develop and correlate diagnostic methods for detection of early nutritional diseases; to determine the significance of various manifestations of nutritional dis-

eases; to determine the most effective methods of treating and preventing deficiency diseases; and to integrate nutrition in other public health problems, such as prenatal, child growth and development, tuberculosis, aging, and degenerative diseases.

To measure the nutritional status and food habits of various population groups, the Bureau plans to institute surveys. As a result, it should be possible to establish a base line observation in critical areas or groups in the city so as to determine the trend of nutritional status. Such base line observations would be of value not only in peacetime planning but in an emergency of either local or national character.

The Bureau of Nutrition has at the present time five clinics, three in Manhattan and two in Brooklyn. Since transportation in Greater New York is fashioned so as to radiate from business centers in Manhattan and Brooklyn, a limited number of clinics can best serve the entire population of the city by being located so as to utilize these means of transportation.

A physician, who is responsible for the medical services rendered, is in charge of each clinic. Assisting him are four to ten physicians. These physicians total thirty at the present time and are paid on a per diem basis.

To each clinic is assigned by the Bureau one of its more experienced nutritionists, full time, who is assisted by one to three other nutritionists during actual clinic sessions.

Also assigned from the Bureau of Nursing to each clinic is a full-time nurse, and an additional conference nurse during each clinic session. Although laboratory technicians have been provided for in the budget, their services have so far not been obtained. In the meantime the following expedients are being used: (1) in two of the clinics the physicians do the necessary laboratory work; (2) in one of the clinics the Bureau of Social Hygiene has shared the services of a technician; (3) in one of the clinics this service has been provided for by a grant from The Nutrition Clinics Fund; (4) finally, the School of Public Health of Columbia University has provided this assistance at another clinic.

In each of the clinics four types of examinations are performed: (1) a thorough medical history and physical examination to detect other diseases and "conditioning" factors which may cause malnutrition in the presence of dietary adequacy; (2) a dietary history to detect unsatisfactory dietary patterns; (3) chemical and instrumental examinations to detect unsatisfactory blood or tissue levels in several of the nutrients, and subgross anatomic changes; and (4) a clinical examination to detect signs presumptive of malnutrition.

The first three of these methods are used primarily for detecting nutritional inadequacies before, not after, clinical signs of malnutrition have developed. Unfortunately, however, many patients referred to the nutrition clinic already have developed one or more signs that may be caused by malnutrition. Since many of these signs represent relatively small and often not obvious deviations from normal, too little significance has been given them in the past. But in malnutrition, as in tuberculosis, the most effective therapy can be given early when the signs are still minimal, therefore the importance of early diagnosis.

The signs of malnutrition in children are similar to those occurring in adults, with certain significant variations: in the first place they are not complicated, as a rule, with signs of chronologic aging; secondly, the signs are often earlier and more acute; finally, general health is probably influenced to a greater extent than in similar nutritional deficiencies in adults.

Although the number of patients seen is not the best or only index of the value of nutrition services to the public, it does provide a quantitative expression that may be

an indication of its acceptance by the community.

The nutrition clinic at the Lower East Side District Health Center was opened in March 1945 and had 1213 clinic visits during that calendar year. In 1946 there were 3159 visits and in 1947, 4988 visits: at this point saturation of facilities was reached so that since 1948 visits have been restricted to approximately 400 per month. This resulted in an impossible situation in that appointments for new patients could only be made after a waiting period of several months. This saturation of facilities at one district health center resulted in three additional clinics in 1949 and one in 1950. During 1950, 3903 individual patients made 15,691 visits.

Of the 3018 new patients seen during 1950, 72 per cent were referred by other services of the Department of Health—chiefly the school health services; 25 per cent were referred by other patients, mostly members of the family group of the original patient; and the remaining 3 per cent were referred by welfare agencies and private physicians.

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THE FEBRILE RESPONSE TO INTRAVENOUS FAT

The potential clinical value of intravenous fat as a major source of parenteral calories is well recognized. Accordingly, the attempts of various laboratories to prepare emulsions of fat suitable for intravenous use in man have aroused widespread interest.

An important barrier to the clinical use of fat emulsions has been the high incidence of febrile responses associated with their administration. Recent publications dealing with this subject have disclosed what appears to be fundamental disagreement about the nature and cause of such febrile responses. G. F. Lambert, J. P. Miller, and D. V. Frost (*Am. J. Physiol.* **164,** *490 (1951)*) have reported on the administration of intravenous fat emulsions to rabbits and dogs. Although they made use of a variety of procedures designed to prevent accumulation of bacterial pyrogens, these investigators were unable to prepare emulsions of fat which passed the U. S. P. XIII pyrogen test (with rabbits). One exception was an emulsion which contained a stabilizer known to inhibit the rate of disappearance of fat from the blood. Lambert and his associates also removed, under aseptic conditions, stored visceral fat from freshly killed rabbits previously treated with penicillin. The rabbit fat was minced and extracted with ether, and made into an emulsion which was then promptly tested for pyrogenicity. It too produced a febrile response.

It was not stated whether homogenization had been accomplished under nitrogen. Oxidation products of fats and oils may conceivably have pyrogenic properties not present in the original unoxidized material. Also, the corn oil used in many of the tests described by Lambert *et al.* is known to contain a number of unsaturated carbon linkages which are particularly subject to oxidation.

Because of their observations the authors proposed the term *thermogenic* as being more appropriate than *pyrogenic* in describing the febrile response to fat administered by vein. They concluded that thermogenicity appeared to be an inherent property of fat emulsions and suggested that the rapid oxidation of intravenously administered fat might produce excessive heat and a concomitant rise in body temperature. This thesis was first proposed by B. G. P. Shafiroff, J. H. Mulholland, and E. Roth (*Proc. Soc. Exp. Biol. Med.* **72,** *543 (1949)*)

as an explanation for the frequent febrile responses observed by them in patients receiving intravenous fat.

In contrast to the observations of Lambert *et al.*, there has been published an article by H. C. Meng (*J. Lab. Clin. Med.* **37,** *222 (1951)*) in which the intravenous administration of fat to rabbits is described. Meng found that injection of amounts of fat comparable to those given by Lambert *et al.* produced no febrile reaction whatever. After several months, however, the emulsions turned "thermogenic."

It is also difficult to reconcile the conclusions of Lambert and his associates with the clinical findings of E. M. Neptune, R. P. Geyer, I. M. Saslaw, and F. J. Stare (*Surg. Gynec. Obstet.* **92,** *365 (1951)*). They administered 64 infusions of a liter or more of a 15 per cent fat emulsion by vein to a series of 9 patients. No rises in temperature were encountered. Turbidity studies indicated that the fat was rapidly cleared from the blood stream. A moderate rise in blood ketones accompanied and followed administration of the fat.

In order to explain the discrepancy between these apparently contradictory reports, it is desirable to examine more closely what is meant by pyrogenicity and thermogenicity. Actually, the word pyrogen implies an agent acting on the temperature regulating centers, primarily to diminish heat loss, but also (as in shivering) to increase heat production. In the introduction to Lambert's paper, pyrogen is described as connoting a bacterial product and the term thermogenicity is reserved for febrile responses to agents *not* elaborated by bacteria. In this context, thermogen is equated with what might with equal accuracy be called a *chemical* (non-bacterial) pyrogen. It has never been demonstrated that pyrogenicity is the exclusive prerogative of bacteria. However, Lambert and his associates conclude that a possible mechanism in bringing about the fever associated with fat infusion is the production of excessive metabolic heat. In other words, the term thermogenic is finally applied by them to all non-bacterial agents which produce fever.

It would appear that a more physiologic distinction between the terms pyrogen and thermogen could be made if pyrogen were used to denote reactions which raise the temperature by producing primarily a disturbance of the temperature regulating centers. Thermogenesis could then be used to designate reactions which raise body temperature primarily by the production of "metabolic" heat. It is perfectly true that mixed reactions usually occur. For example, epinephrine increases heat production; however, it acts on temperature principally by decreasing heat loss (T. Sollmann, "A Manual of Pharmacology," *W. B. Saunders, Philadelphia, p. 582 (1942)*). In contrast, dinitrophenol exerts its effect on body temperature by increasing oxidative metabolism and heat production in the periphery.

The effect of dinitrophenol is not prevented by extirpation of the heat regulating centers, nor by curare. (P. van Uytvanck, *Arch. internat. de Pharmacodyn. et de Thérap.* **41,** *160 (1931)*). On the other hand, the injection of bacterial products in decerebrate animals does not cause fever (C. H. Best and N. B. Taylor, "The Physiological Basis of Medical Practice," *Williams and Wilkins Co., Baltimore, p. 1604 (1943)*).

In summary, it may be said that two general types of febrile response can be postulated to explain the fevers which have been observed following administration of fat. One response appears to be mediated chiefly via the temperature regulating centers, the other occurs directly in the periphery by virtue of an increase in oxidative metabolism. Accordingly, the terms pyrogenic and thermogenic would appear to be of value only if they are given a physiologic rather than an etiologic meaning.

In the work of Lambert *et al.*, only bacterial pyrogens seem to have been ruled out. However, no convincing evidence has been brought forward by them to indicate the

physiologic source of the febrile response in the rabbits studied. It is regrettable that they did not study a group of rabbits in which the heat regulating centers had been previously destroyed.

The observations of Neptune and his associates indicate that intravenously infused emulsions of fat actually do not produce excessive peripheral heat. Oxygen consumption in a volunteer studied by Neptune and associates during and after fat administration did not increase remarkably. Apparently the metabolic rate must be doubled before antifebrile compensations are overcome (Sollmann (*loc. cit.*)). Previous studies with intravenous fat emulsions (G. V. Mann, R. P. Geyer, D. M. Watkin, and F. J. Stare, *J. Lab. Clin. Med.* **34,** *699* (*1949*)) have described febrile responses to fat involving a chill followed by fever. This is not a characteristic of metabolic hyperthermia, and from the C^{14} studies of R. P. Geyer, J. Chipman, and F. J. Stare (*J. Biol. Chem.* **176,** *1469* (*1948*)) in rats, there is every reason to suppose that intravenously infused emulsions of fat used by Neptune are fully capable of being rapidly oxidized.

Further evidence for the non-intrinsic nature of thermogenicity of intravenous fat is in the report of Mann *et al.* (*loc. cit.*) describing clinical fat emulsions which were non-pyrogenic for a period of a month or more but which later "turned" pyrogenic.

It must then be concluded from the evidence presented in these apparently contradictory papers that the thermogenic response described by Lambert *et al.* is in all probability the physiologic analogue of the bacterial pyrogen response. In any case, the observations of Neptune and of Meng have conclusively demonstrated that intravenous fat per se is by no means a thermogenic agent.

Certainly it is not permissible fully to equate responses in rabbits with responses in man. But what should be stressed is that, regardless of the response in the rabbit, the administration of intravenous fat in man seems to have been established as a feasible clinical procedure.

Further work remains to be done. It should be demonstrated by isotope studies that oxidation of intravenously administered fat takes place at a fairly constant rate whether a febrile response is elicited or not. Failure of a decerebrate rabbit to respond with fever to fat of known pyrogenicity would favor the concept that nervous factors play the greater role in fat-induced fever.

From a practical standpoint, the view that fat is intrinsically thermogenic would tend to discourage further work on the development of clinical fat emulsions. The facts do not appear to warrant such pessimism. Indeed, the findings of Neptune and associates indicate that the problems to be overcome in the manufacture of fat emulsions are technical and not physiologic.

AN UNIDENTIFIED SUBSTANCE WITH VITAMIN A ACTIVITY

Because of the importance of vitamin A in the diet of all higher animals, the chemical nature of this vitamin was quite early determined to be that of an unsaturated primary alcohol containing four isoprene residues, two of which were present as an unsubstituted β-ionone ring. It was also recognized that a number of plant pigments—the so-called carotenoid pigments—such as α- and β-carotenes, cryptoxanthine, etc., possessed vitamin A activity. Activity of the latter compounds results from the fact that a portion of their molecules is identical in structure to the carbon skeleton of vitamin A itself, and the animal body is able to transform them, at least in part, into vitamin A.

The presence of each of these substances possessing vitamin A activity is rather

readily detected in oily materials due to the fact that they are all found in the non-saponifiable portions of such materials and possess characteristic absorption spectra. Many of them also yield characteristic colors on treatment with antimony trichloride.

A recent article by H. Kaunitz and C. A. Slanetz (*J. Nutrition* **42,** *375 (1950)*) is of great interest because it indicates the possible existence of one or more substances with vitamin A activity that differ significantly from the above group of compounds in properties. Freshly rendered lard was subjected to molecular distillation at 215° under very high vacuum. Seven per cent of the lard distilled under these conditions. This volatile portion will be called the distillate. This procedure normally removes vitamins A and E from vitamin-bearing oils. Spectrophotometric examination of the non-saponifiable portion of this distillate, however, revealed the presence of not more than 0.1 microgram of vitamin A per gram of distillate; no vitamin A whatsoever was detected in the distillate by the antimony trichloride test. The distillate did contain approximately 15 mg. of tocopherols (vitamin E) per 100 g.

Various levels of vitamin A were then given to rats fed a vitamin A-free ration generously supplemented with the other vitamins. Excellent growth and survival responses to vitamin A were obtained, proving the suitability of the ration and of the method of administration of vitamin A. Several comparisons of the effect of the distillate and of vitamin A were then made. In all cases, feeding of 2 per cent of the distillate in the diet was very significantly superior to administration of 1.25 units weekly of vitamin A palmitate in promotion of growth, in the prevention of symptoms of vitamin A deficiency, and in promoting survival of the animals. To cite only one of several equally convincing examples, all of the vitamin A controls were dead at 67 days of age (starting age on diet: 21 to 28 days), whereas none of the distillate group had died, and all of the latter group were still growing even after eight weeks on the diet. This level of administered vitamin A is higher than would be supplied by the distillate, assuming even the highest of the experimentally determined values for its vitamin A content to be correct. Indeed, 2 per cent of the distillate promoted better growth and kept the animals in better condition than administration of 7.5 units of vitamin A weekly.

To check the possibility that the distillate acted by supplying substances which promoted much more efficient utilization of preexistent stores of vitamin A, 19 rats were kept on the deficient diet without supplement from weaning to an age of 45 days. At this time, growth had ceased and severe symptoms of vitamin A deficiency (ocular lesions, roughened fur, etc.) were evident. They were then separated into two groups, one of which received 2 per cent of distillate, while the other received 2.5 units weekly of vitamin A. After thirty-eight days on the diet, 5 of the distillate group were alive and growing well; all of the animals receiving the vitamin A were dead. The experiment demonstrates that the distillate was highly effective in promoting growth and alleviating symptoms of vitamin A deficiency even in severely depleted animals.

After full consideration of possible alternative explanations for their results, the authors conclude that the distillate fraction from lard contains a factor with vitamin A-like activity, but which is chemically distinct from vitamin A itself, or from any of the known compounds possessing vitamin A activity. The conclusion appears fully justified by the results reported.

If such a factor actually is present in animal fats, it adds additional uncertainties to the results of bioassays for vitamin A, and casts some doubt upon the meaningfulness of figures for the vitamin A content of natural materials determined by chemical and physical methods. The distribution of the

substance is not yet known. Elucidation of its chemical nature, and the biological basis for its effectiveness, may go far to explain the role of vitamin A in cellular physiology, a problem which has scarcely been broached at present.

ATHEROSCLEROSIS, SERUM CHOLESTEROL, AND LIPOPROTEINS

A relationship between the level of serum cholesterol and atherosclerosis has been thought to exist because of (1) the presence of large amounts of cholesterol in atherosclerotic lesions, (2) the production of experimental "atherosclerosis" in animals by inducing hypercholesterolemia, (3) the high prevalence of atherosclerosis which is said to exist in persons who have diseases associated with hypercholesterolemia, *i.e.*, diabetes mellitus, myxedema, nephrosis, and essential familial xanthomatosis, and (4) the finding of elevated levels of serum cholesterol in patients with coronary artery disease. Because the relationship between atherosclerosis and the presence of an elevated serum cholesterol is not very predictable, various cholesterol fractions and blood lipids have been studied in an attempt to find a better correlation.

Recent reports by J. W. Gofman and associates (*Circulation* **2,** *161 (1950)*; *Science* **111,** *166 (1950)*; *Nutrition Reviews* **8,** *176 (1950)*) imply that there is a better correlation between atherosclerosis and the presence and concentration of certain cholesterol-containing molecules in the serum, than between atherosclerosis and the level of total serum cholesterol. They have developed a method, utilizing the ultracentrifuge, by which certain groups of lipoprotein and lipid molecules may be identified according to their densities, and their concentrations measured. The molecules are classified according to their flotation rates in the ultracentrifuge. The following classes of molecules have been described in human sera: (1) molecules with flotation rates greater than 75 S_f units which include chylomicrons, represent part of the alimentary lipemia, and are said not be correlated with atherosclerosis, (2) molecules with flotation rates of 30 to 70 S_f units which contain some cholesterol, represent part of the alimentary lipemia, and whose correlation with atherosclerosis is under study, (3) molecules with flotation rates of 10 to 20 S_f units which contain cholesterol and are said to be correlated with atherosclerosis, and (4) molecules with flotation rates of 3 to 8 S_f units which contain most of the serum cholesterol and which are said not to be correlated with atherosclerosis.

Initial studies were made of the sera of rabbits who were fed 3 g. of cholesterol a week for fifteen weeks. There was an initial rise in the concentration of molecules of the S_f 5 to 8 class (corresponding to the S_f 3 to 8 class in man) up to four times their original concentration. In most animals, a new class of cholesterol-containing molecules with flotation rates of 10 to 30 S_f units then appeared, and this rapidly increased in concentration. At the end of fifteen weeks the animals were autopsied and the presence and severity of "atherosclerosis" were noted. The animals which failed to develop high concentrations of molecules of the S_f 10 to 30 class showed no gross or only minimal atherosclerosis, while in the remaining animals the degree of atherosclerosis seemed to correlate roughly with the concentration of these molecules. While data on the level of the total serum cholesterol of these animals are not given, it is evident that the animals which developed high concentrations of S_f 10 to 30 molecules must have developed marked hypercholesterolemia. This is consistent with previous work which has shown that animals which fail to develop hyper-

cholesterolemia also fail to develop atherosclerosis. Data are reported for only 12 animals, a group too small for useful statistical analysis.

Studies are also reported for humans, sera from 1553 persons having been analyzed. Apparently all sera contained molecules with flotation rates of 3 to 8 S_f units and of 30 or more S_f units. In many sera the presence of molecules with flotation rates of 10 to 20 S_f units was found, and study was made of their incidence and concentration in the sera of "healthy" persons and of persons with various diseases. After study of the sera of patients who had in the past had a myocardial infarction, the authors felt that there was "unequivocal evidence that the presence of these molecules is in some way associated with the presence of atherosclerosis."

Three principal questions should be asked about this work:

(1) Is the accuracy and reliability of this new method sufficient to answer the problem being studied?

(2) Were the subjects studied chosen in such a way that data derived from analysis of their sera might answer the problem being studied?

(3) Do the data support the authors' conclusions and implications?

The first question cannot be answered since no data are presented by the authors or have been published elsewhere about the accuracy or reliability of the method.

In regard to the second question, the following persons were studied: (1) 869 "healthy" men and 258 "healthy" women, (2) 230 men and 32 women who had in the past had a myocardial infarction, and (3) various numbers of individuals with diabetes mellitus, angina pectoris, hypertension, myxedema, nephrosis, and miscellaneous conditions. The "healthy" subjects were 20 years of age or over and had no known disease. No details are given as to the source of these persons. The patients with myocardial infarcts were referred to the authors by a number of physicians. These patients were included when there was (1) a typical clinical history of myocardial infarction, (2) conclusive laboratory evidence such as an elevated sedimentation rate, leukocytosis, and fever, and (3) electrocardiographic changes typical of myocardial infarction. It was believed that 95 per cent or more of these patients had atherosclerosis. No information is given about the selection of these patients; *i.e.*, were they all the patients with myocardial infarction seen by the referring physicians? Did they constitute a random sample? Were their serum cholesterol levels known to the referring physicians, and did these influence their selection? This latter point is very important, for as will be shown, there is a strong positive correlation between the level of serum cholesterol and the concentration of molecules of the S_f 10 to 20 class.

The third question can be answered from analysis of the data, which is presented in the form of scatter diagrams for the various clinical groups. Comparison of the data for 869 "healthy" men and 230 men with myocardial infarcts shows a significantly higher incidence and concentration of molecules of the S_f 10 to 20 class in the patients with coronary disease. These molecules occurred in 68.7 per cent of the "healthy" men and in 94.2 per cent of the patients. This difference is statistically significant, the significance ratio being 7.09. The concentration of these molecules in "healthy" men was 17.8 mg. per cent, and in the patients, 36.5 mg. per cent (significance ratio 10.6). The same differences can be observed when the data are analyzed in ten-year age groups. The data for women with myocardial infarcts are few, but suggest that the same differences exist for the women. Thus the patients in this study who had a myocardial infarct did have a higher incidence and concentration of molecules of the S_f 10 to 20 class than did the "healthy" controls. Further analysis throws some doubt on the meaning of this difference.

Data are also presented in the form of

scatter diagrams concerning the relation between the level of serum cholesterol and the concentration of molecules of the S_f 10 to 20 class in 89 "healthy" men, 97 men who had a myocardial infarct, 58 patients with diabetes, and 30 persons who had hypercholesterolemia associated with various conditions. This enables one to (1) determine the correlation coefficient between the level of serum cholesterol and the concentration of molecules of the S_f 10 to 20 class, and (2) compare the incidence, mean concentration and distribution curves of serum cholesterol and these molecules.

The correlation coefficient between the level of serum cholesterol and the concentration of molecules of the S_f 10 to 20 class in all 274 sera is plus 0.55. (Significance ratio 9.02). This correlation is highly significant, but not very predictable. The correlation is also significant in each of the smaller groups.

Molecules of the S_f 10 to 20 class were found in 96.4 per cent of the 97 men with a myocardial infarct, and in 64.1 per cent of the 89 "healthy" men (significance ratio 5.67). However, serum cholesterol levels greater than 200 mg. per cent were found in 92.9 per cent of the patients and in 56.2 per cent of the "healthy" men (significance ratio 5.87). Thus the presence of molecules of the S_f 10 to 20 class was no more closely associated with atherosclerosis than was a serum cholesterol level greater than 200 mg. per cent.

The mean concentration of molecules of the S_f 10 to 20 class was 36.33 mg. per cent in men with myocardial infarcts and 18.27 mg. per cent in "healthy" men (significance ratio 4.65). The mean serum cholesterol level was 258.6 mg. per cent for the patients and 207.1 mg. per cent for "healthy" men (significance ratio 6.94). Thus the difference in mean concentration of molecules of S_f 10 to 20 class between "healthy" men and men with myocardial infarcts is no more significant than the difference in the mean serum cholesterol level between the same persons.

Distribution curves for these molecules and for serum cholesterol in the "healthy" men and men with myocardial infarcts are quite similar.

Thus in all respects, the serum cholesterol seems to be as closely correlated with atherosclerosis as does the S_f 10 to 20 class of molecules. Furthermore, the serum cholesterol is closely correlated with the S_f 10 to 20 class of molecules. The authors' statement that serum cholesterol does not correlate well with these molecules is not supported by the data, nor is the implication that these molecules correlate better with atherosclerosis than does serum cholesterol.

An increased incidence and concentration of molecules of the S_f 10 to 20 class was also noted in patients with diabetes mellitus, nephrosis and myxedema, which the authors argue is additional evidence of the association of these molecules with atherosclerosis. This is primarily evidence of their association with hypercholesterolemia which is common in these conditions.

This analysis does not indicate that these molecules are not related to or even the cause of atherosclerosis, but it does indicate that such remains to be proved. This work represents a new approach to an important problem. The questions raised can be answered by careful evaluation of the method, selection of the persons to be studied, and evaluation of the results.

RESISTANT RICKETS; FAMILIAL OCCURRENCE

The vitamin D deficiency syndrome known as resistant or refractory rickets has been well described previously but only limited numbers of cases have been reported (*Nutrition Reviews* **2,** *218 (1944)*; **6,** *79 (1948)*; **8,** *221 (1950)*). A recent report, re-

viewing experience in 25 cases in children and 10 adult patients at the Shriner's Hospital for crippled children in St. Louis indicates that the number of patients with this disease may be greater than commonly estimated (H. E. Pedersen and H. R. McCarroll, *J. Bone Joint Surg.* **33A,** *203 (1951)*). The series was compiled by reassaying patients suspected of the diagnosis in retrospect, many of whom had been followed but previously misclassified, during a seventeen-year period. It is significant that the children were patients being followed in an orthopedic clinic where they had had or were undergoing reparative surgery for bone deformities. The 10 adults were discovered among relatives of the children.

Of the children, 16 were found to be distributed among 10 family groups in which one parent had similar deformities and characteristic histories. Extensive exploration of the family histories revealed a total of 22 relatives with probable rachitic deformities. The extent of occurrence in families is indicated by the following examples: one patient had 8 relatives with involvement, 2 patients who were siblings had 4 relatives with involvement and another patient had 3 relatives with suggestive deformities. In the series, 4 families were found with 2 rachitic children and one or more relatives deformed, and one family had 3 of 8 living children and the mother diagnosed as resistant rickets sufferers.

The 25 patients reported had histories of rickets beginning in childhood despite the administration of usually adequate doses of vitamin D (25 to 75 drops of cod liver oil concentrate daily or cod liver oil with percomorph oil, 3 to 4 teaspoonfuls a day). Deformities of the typical variety had developed however, and 97 osteotomies for repair of these had been done, and an additional 25 had been carried out for recurrences of the deformity in previously operated extremities. No gastrointestinal or renal disease was found in the patients, 20 of whom had received extensive study of blood chemistry, urinalyses, and x-ray examination. Of the remainder, all had been surveyed by history, physical examination, and x-ray, and two had had urine studies. Eight patients were investigated while hospitalized and 17 were studied while outpatients.

Of the 10 adults who were parents of the patients, 9 had received similar extensive study as outpatients while the tenth was included on the basis of history. Five of these previously had surgical corrective procedures and 3 others had severe deformities with no history of operations. It is of interest that the authors state that recurrences of deformities have been noted during adult life (over 15 years of age in their clinic). This is in contrast to the usual experience in rickets that deformities do not occur after epiphyseal closure. Short stature, attributed primarily to poor growth of lower extremities, was noted as a striking feature of the adults. Among this group 6 had shown progression of their disease after the age of epiphyseal closure, and symptoms of easy fatigability, inability to perform hard work, and pain in the back, hips and knees were commonly noted.

The usual bone findings of coarse, widely-spaced trabeculae and epiphyseal widening were seen in the children on x-ray examination. Chemical abnormalities were chiefly those of low serum phosphorus, low or normal serum calcium, and elevated alkaline phosphatase. Serum phosphorus was below 3.3 mg. per cent in 23 patients and alkaline phosphatase was 20 Bodansky units or above in 12 patients, 15 to 20 in 4 others, and 12 units or less in 7. The latter group with relatively normal phosphatase values were all 13 years of age or over at the time of study, which may have indicated decreasing bone forming activity at the age of epiphyseal closure. Urine Sulkowitch tests performed on 23 patients showed negative to trace values in all.

In the 9 parents and one other adult who were studied, serum phosphorus was low in 8 (maximum of 2.5 mg. per cent) and two others had 3.7 and 4.2 mg. per cent respectively. Alkaline phosphatase was 6 Bodansky units or below in all subjects and serum calcium varied from 8.8 to 11.4 mg. per cent. No figures for total protein were given by the authors for correlation with the serum calcium values in either adults or children, so that it must be assumed that no marked change in free or bound calcium was discernible.

The experience of the authors in attempting therapy in a series of resistant rickets of such relative magnitude as this is worthy of note. Two findings consistent with the authors' experience stand out: (1) improvement in the appearance of the epiphyseal lines on x-ray after postoperative plaster immobilization without vitamin D and (2) the occurrence of vitamin D intoxication (or hypercalcemia) symptoms when large doses of the vitamin were given to patients immobilized in casts. Epiphyseal line improvement was noted during plaster immobilization not only in the bones subjected to osteotomy but improvement to the same degree occurred in all visible epiphyses. It is stated that every patient on whom adequate studies were made demonstrated such improvement but the number of such patients is not included.

In 7 patients vitamin D was administered during the operative and cast immobilization period with resultant anorexia, nausea, vomiting and weight loss in all. Unfortunately no data on serum calcium and phosphorus of Sulkowitch tests during this episode were reported, but cessation of vitamin therapy was followed by relief of these typical symptoms of hypercalcemia. Several of these patients were subsequently given similar amounts of vitamin D while ambulatory and were then able to tolerate it without symptoms. The mechanism of healing during immobilization and of hypercalcemic symptoms during the combination of immobilization and vitamin D administration would appear to be related and perhaps explainable on the basis of Albright's finding that immobilization in children may lead to an increase in serum calcium and phosphorus levels (F. Albright and E. C. Reifenstein, "The Parathyroid Glands and Metabolic Bone Disease," *Williams and Wilkins Co., Baltimore, p. 85, (1948)*). Thus hypercalcemia of immobilization would then be increased still further by the improvement in calcium absorption provided by vitamin D.

The authors report that 17 patients were under therapy with 100,000 to 600,000 units of vitamin D daily at the time of the report. X-ray evidence of healing occurred in all subjects followed sufficiently long during treatment. The relatively short duration of their studies, however, precludes any conclusions as to the end results of adequate vitamin administration, and the writers emphasize both the variability in requirements and the relatively narrow range between therapeutic and toxicity-producing doses. In their experience, maintenance of a one- to two-plus Sulkowitch test in the urine provides a reasonable objective in therapy but must be accompanied by repeated serum calcium and phosphorus determinations.

This report suggests that the diagnosis of resistant rickets may be missed in many cases due to confusion with various syndromes leading to dwarfism and that the familial characteristics have not been sufficiently recognized by clinicians. Family involvement has been previously reported in isolated instances by R. Lightwood and I. J. Wood (*Proc. Roy. Soc. Med.* **26,** *277 (1933)*) and by A. M. Gill (*Arch. Dis. Child.* **14,** *50 (1939)*) and others. Two problems worthy of investigation are suggested by these studies: a clarification of the genetics of the disease, and a study of the incidence of resistant rickets among patients in large orthopedic clinics with such diagnoses as achondroplasia and chondroosteodystrophy.

EFFECT OF INTERACTION WITH GLUCOSE ON THE NUTRITIVE VALUE OF PROTEIN HYDROLYSATES

The nature of the chemical reactions that amino acids undergo upon long-continued contact with reducing sugars such as glucose has not been entirely clarified. The importance of such reactions to the food industry has become increasingly clear during the past several years, inasmuch as the reaction is now known to be the cause of the non-enzymatic browning of foodstuffs. Although the latter reaction is responsible for much of the general deterioration (discoloration and lowered palatability) that may occur during processing and storage of food materials, its influence on the nutritive quality of food proteins has not been emphasized until recently. In 1948, however, A. R. Patton, E. G. Hill, and E. M. Foreman (*Science* **107,** *68, 623 (1948)*) showed that autoclaving certain amino acids with glucose-containing media decreased their availability to certain lactic acid bacteria used for the assay of these amino acids.

That the same result is obtained in animals, and to a much greater extent, appears from the recent results of L. Friedman and O. L. Kline (*J. Nutrition* **40,** *295 (1950)*). The protein preparation chosen for investigation was a partial pancreatic hydrolysate of casein of the type used for oral feeding and for preparation of solutions for parenteral administration. Concentrated aqueous solutions of this preparation were mixed with glucose, the solutions concentrated to a small volume in a vacuum still and subsequently dried on dextrin under various conditions of time and temperature, usually involving exposure to 50 to 60° C. for thirty-six to sixty hours. The latter preparations were then used as the sole source of amino acids in a protein-free diet for rats, and their ability to support growth was compared with that of control samples dried under similar conditions, but without glucose. Losses of nutritive value due to drying with glucose ranged from about 18 to 100 per cent in the various preparations, depending upon the temperature and the duration of heating. When preparations with reduced growth-promoting ability were supplemented with protein of high quality, essentially normal growth resulted, showing that failure of the treated hydrolysates to support growth resulted from actual loss in nutritive value, rather than from production of toxic materials.

To determine the amino acids primarily affected by drying with glucose, combination of the glucose-treated protein hydrolysates and various essential amino acids were fed. Neither tryptophan nor methionine, individually, restored growth-promoting activity to the treated hydrolysate; a mixture of the nine essential amino acids, however, was effective. By deleting individual amino acids from this mixture, it was determined that histidine, threonine, phenylalanine, tryptophan, lysine, and methionine all were adversely affected by the glucose treatment. The other essential amino acids were damaged to a lesser extent.

Microbiologic assays of the glucose-treated hydrolysates confirmed in a general way the results of animal assay, *i.e.*, the same amino acids that were indicated by animal assay to be low were also lower by microbiologic assay in the glucose-treated casein hydrolysates than in the untreated hydrolysates. Good correlation also existed between the extent of destruction indicated by the two methods. The authors express the conviction, however, that the extent of destruction of the essential amino acids, as indicated by microbiologic assay, was insufficient to account for the pronounced effects on nutritive value for the rat. For example, the glucose-treated hydrolysate which lost 100 per cent of its nutritive value for the rat showed losses in these various critical amino acids of only 18 to 30 per cent as

indicated by microbiologic assay. It thus appeared that an important difference in availability of the amino acid-glucose reaction products existed for the microorganism (*Streptococcus faecalis*) and the rat, the products being much less available to the latter organism.

The significance of these results for the food manufacturer and the practical nutritionist is not yet apparent. Although the relatively drastic conditions used here to demonstrate the nutritive effects of the reaction would seldom occur in the processed food industry, smaller changes in the same direction undoubtedly do occur, and might well be of concern in some cases. Knowledge that the effect exists is, of course, the first prerequisite to attempts to minimize any possible deleterious effects on the nutritional value of food products.

PARENTERAL ADMINISTRATION OF FRUCTOSE

Dextrose (glucose) is the most commonly used nutrient in intravenous therapy; yet its value as a source of calories by this route is somewhat limited. Retention of an intravenously administered sugar is ordinarily a function of two variables, the ability of the body to remove the substance from the blood, and its renal threshold. Glucose is taken out of the blood at a rate which permits its efficient utilization when it is given by mouth. When it is given by vein, however, a variable amount is lost in the urine depending on the rapidity of administration. In addition, the glucosuria which ensues is accompanied by a diuresis which in itself may be detrimental.

Accordingly, it has been found desirable to limit the rate of dextrose administration to approximately 0.5 g. per kilogram per hour (S. B. Winslow, *Surgery* **4,** *867* (*1938*)). In concentrations exceeding 5 per cent, glucose solutions are damaging to veins and roughly in direct proportion to their hypertonicity. Therefore it is ordinarily necessary to administer glucose in a dilute solution along with volumes of fluid which may, under certain circumstances, be dangerous to the patient. These limitations have led to the search for more satisfactory calorigenic preparations.

The tolerance of human subjects to an intravenously administered mixture of glucose and fructose (invert sugar) has been under investigation (J. J. Weinstein, *Med. Ann. District of Columbia* **19,** *179* (*1950*)). Ten patients in the fasting state received a liter of 5 per cent invert sugar (containing equal parts of glucose and fructose) in isotonic saline. The material was injected at an average rate of 1.5 g. per kilogram per hour, and under these conditions, less than 1 per cent of the infused sugar was lost in the urine. This was in distinct contrast to the 6 per cent loss which followed administration of an equivalent amount of glucose at the slower rate of 0.78 g. per kilogram per hour to the same group of subjects. Later, 12 patients received 10 per cent invert sugar in water at an average rate of 1.5 g. per kilogram per hour with a urinary loss of less than 2 per cent (Weinstein, *Ann. Western Med. Surg.* **4,** *373* (*1950*)).

Fructose levels in blood and urine were not determined in the invert sugar studies. Recently, however, tolerance to intravenous fructose alone has been reported by T. E. Weichselbaum, R. Elman, and R. H. Lund (*Proc. Soc. Exp. Biol. Med.* **75,** *816* (*1950*)). Comparative studies are described in which a liter of 10 per cent glucose and 10 per cent fructose in water, respectively, were administered by vein on separate days to each of 10 human subjects. The fructose was injected at rates ranging from 0.7 to 2.0 g. per kilogram per hour. The glucose never was given more rapidly than 1.1 g. per kilogram per hour. It was found that the values for fructosuria following fructose ad-

ministration were relatively low, at no time exceeding 6 per cent of the amount given. At the same time, the glucosuria which followed administration of glucose was very much higher. Blood fructose levels rose only moderately during the period of fructose injection.

Earlier studies on the renal threshold for fructose in rats (C. F. Cori and G. T. Cori, *J. Biol. Chem.* **72,** *597 (1927)*) and in man (R. T. Woodyatt, W. D. Sansum, and R. M. Wilder, *J. Am. Med. Assn.* **65,** *2067 (1915)*) have shown that fructose is far less readily retained by the kidneys than is glucose. Accordingly, the recent clinical findings of T. E. Weichselbaum and his associates suggest that the intravenously administered fructose was removed from the circulation at a rate precluding its appreciable loss in the urine.

At the same time, it should be pointed out in the case of invert sugar that when two different sugars are administered together, the resulting excretion of sugar in the urine will be determined by the separate blood levels of the sugars, not by the sum of their concentrations in the blood. Each saccharide is treated separately by the kidney; accordingly, from the standpoint of renal "threshold," the injection of 10 per cent invert sugar may be better regarded as an injection of two 5 per cent sugar solutions, one fructose and the other glucose.

No work has yet been reported concerning tolerance of human subjects to repeated injections of fructose. E. P. Joslin has described the use of oral fructose in diabetic individuals ("The Treatment of Diabetes Mellitus," *Lea and Febiger, Philadelphia, 8th Ed., p. 356 (1946)*). At first the fructose seemed to be well utilized, but gradually more and more glucosuria ensued and eventually no advantage of fructose over glucose could be observed. Similarly, in their studies on pancreatectomized dogs, J. L. Bollman and F. C. Mann (*Am. J. Physiol.* **107,** *183 (1934)*) found that fructose was partly utilized for brief periods. However, the beneficial effect was temporary and eventually most of the administered fructose appeared in the urine as glucose. One mild diabetic in the series studied by Weichselbaum *et al.* tolerated the injected fructose well, with blood fructose levels that compared favorably with the values found in nondiabetic subjects under similar experimental conditions.

S. S. Chernick and I. L. Chaikoff (*J. Biol. Chem.* **188,** *389 (1951)*) have studied the utilization of added C^{14}-glucose and C^{14}-fructose by surviving slices from normal and alloxan-diabetic rats. Their results suggest the possibility of preferential utilization of fructose by normal hepatic tissue. Moreover, when C^{14}-labeled fructose was added to the medium in their experiments with diabetic liver slices, $C^{14}O_2$ recoveries were normal. At the same time it was shown that the conversion of added glucose to CO_2 was impaired in diabetic liver slices. Although the oxidation of fructose C^{14} was normal in the diabetic liver, lipogenesis from both fructose and glucose was depressed to about the same level.

It has also been shown that the comparative phosphorylation rates of fructose and glucose in rat liver slices and homogenates are very different, the fructose apparently being far more readily phosphorylated than glucose (C. S. Vestling, A. K. Mylroie, U. Irish, and N. H. Grant, *J. Biol. Chem.* **185,** *789 (1950)*). If phosphorylation is a limiting factor in the rate of metabolic utilization of hexoses, the relative ease of phosphorylation of fructose may explain the rapidity with which it disappears from the blood stream.

The rise in RQ that follows administration of fructose has been attributed to rapid formation of lactic acid which in turn induces liberation of CO_2 from bicarbonate (W. R. Campbell and E. J. Maltby, (*J. Clin. Invest.* **6,** *303 (1928)*). An alternative explanation, however, is that the RQ response is due in part to rapid conversion in the liver of carbohydrate to fat. The evidence on this point is conflicting.

In evaluating fructose and invert sugar as sources of parenteral calories, it must be remembered that the rapid administration of parenteral sugar solutions for caloric purposes is rarely indicated. Moreover, the sudden increase in plasma volume which ensues can actually precipitate cardiac failure in patients in borderline cardiac compensation.

Although the difference in tolerance rates between glucose and invert sugar may appear to be appreciable when the two are compared, the *caloric* difference is not impressive. It is difficult to attach any nutritional significance to differences in urinary excretion of sugar of 5 to 10 g. representing caloric loss or gain to the patient of only 20 to 40 calories.

Should fructose prove to be calorically interchangeable with glucose, its role in parenteral feeding would then be determined in part by such considerations as expense to the patient and, in part, by the individual clinical problem. Perhaps in some situations demanding rapid administration of sugar with optimal utilization, fructose and invert sugar may ultimately fill a definite clinical need. The preliminary work of Weinstein has been a stimulant to further elucidation of the fructose problem.

CLINICAL STUDIES ON HYPOTHIAMINOSIS IN MIDDLE-AGED AND ELDERLY INDIVIDUALS

Nutrition surveys carried out in Bataan (Philippines) by H. B. Burch *et al.* (*J. Nutrition* **42,** *9 (1950)*; *Nutrition Reviews* **9,** *141 (1951)*) indicated that, on the average, the values of blood thiamine were progressively lower in groups of individuals ranked according to the increasing severity of recognized symptoms and signs of beriberi. The mean for the nonsymptomatic individuals (N = 50) was 4.0 micrograms of thiamine per 100 ml. of blood; for doubtful cases (N = 68), 3.7 micrograms; for individuals suspected of having beriberi (N = 52), 3.4 micrograms; and in persons with frank beriberi (N = 20), 3.2 micrograms.

Statistically, the difference between the mean blood thiamine values of individuals with frank beriberi and the nonsymptomatic group was highly significant. For the clarification and diagnosis of beriberi in individuals the thiamine level in the blood is not an adequate criterion. Thus in the sample of cases with frank and suspected beriberi 40 per cent had values below 3 micrograms per 100 ml.; at the same time about 30 per cent of the doubtful cases and 20 per cent of nonsymptomatic individuals had thiamine values below 3 micrograms.

In middle-aged and elderly individuals on an apparently adequate food intake the relationship between the level of thiamine in the blood and incidence of clinical symptoms was investigated by J. E. Kirk and M. Chieffi (*J. Gerontol.* **5,** *236 (1950)*).

The subjects, 50 years old and older, were nmates of the St. Louis Infirmary. Patients with hypertension, cardiac failure, and with diseases which might be expected to produce symptoms generally ascribed to thiamine deficiency were not included. There were 42 patients with low blood thiamine levels (below 2.0 micrograms per cent) and 26 individuals with normal thiamine concentration in the blood (above 3.5 micrograms per cent, with a mean age of 71 and 68 years, respectively).

In a larger group of subjects (N = 249) studied earlier and including a greater number of young adults, a mean value for blood thiamine was 3.4 micrograms (*J. Nutrition* **38,** *353 (1949)*).

The dietary intake of thiamine was not studied with precision. On the average, the diet in the institution was calculated to contain 1.9 mg. of thiamine per day. The direct determination of the thiamine content

of the prepared food yielded a somewhat lower value, 1.5 mg.

The occurrence of over fifty symptoms and signs in the two groups was tabulated and a calculation of the statistical significance of the differences was made. The characteristics with a critical ratio of over 2.0 included general fatigue, weakness and heaviness of legs, pain in legs, redness and fissures of tongue, inflammation of the eyelids and conjunctiva (blepharoconjunctivitis) and conjunctival thickening, impaired temperature and vibratory sense in the lower extremities, impaired ankle reflexes, tenderness of calves, and edema of the legs. These symptoms were found with greater frequency in the subjects with a low thiamine concentration in the blood than in the subjects with "normal" blood levels.

The picture is in general consistent with the syndrome observed in subjects with experimentally produced depletion of vitamins of the B-complex (*see* e.g., M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman, *National Res. Council Bull.* **116** (*1948*); *Nutrition Reviews* **7**, *264* (*1949*)).

The positive results always have a greater appeal. However, the negative results obtained in the study by Kirk and Chieffi should not be neglected. Several of them are unexpected. Thus there was no differential impairment of appetite; the incidence of this symptom was identical in both groups and it was low (8 per cent).

As noted above, the patients with low blood thiamine levels had a higher incidence of edema. However, there was no significant difference in the heart size, while cardiac enlargement is present in clinical beriberi and was noted in long-continued experimental thiamine deficiency (H. R. Street *et al.*, *Yale J. Biol. Med.* **13**, *293* (*1941*)). Also, the pulse rate in the two groups showed no significant difference. The edema of the legs was not associated with a reduction in the plasma protein content. The values for both groups were within normal limits.

Several of the neuromuscular findings were also negative. In a separate study (M. Chieffi and E. Kirk, *J. Gerontol.* **4**, *127* (*1949*)) it was shown that motor chronaxie measurements offer little help in the clinical diagnosis of moderate thiamine deficiency. Out of a large number of reflexes studied, only the impaired ankle jerk tended to differentiate the subjects with a low and normal thiamine content in the blood.

Babinski's sign (extension of the toes instead of flexion on stimulating the sole of the foot) was somewhat more frequent in the group with low blood thiamine levels, and the Romberg's sign (marked swaying of the body when standing with the feet close together and the eyes closed) was somewhat more frequent in the group with normal thiamine levels; the differences did not reach statistical significance.

The data on the men with normal thiamine content, indicating a high incidence of some of the symptoms which might be interpreted as signs of thiamine deficiency, are instructive. Thus impairment of vibratory sense in the upper extremity, tested with a stimulus of high frequency (512 vibrations per second), was present in 42 per cent of the "normal" subjects; in the lower extremity, in 83 per cent; the incidence percentages in the group with low thiamine levels were 69 and 73 per cent.

Impaired upper abdominal reflex occurred somewhat more frequently in the group with normal thiamine levels (46 versus 42 per cent); impairment of the lower abdominal reflexes was present in 48 per cent of the patients with normal thiamine levels and in 54 per cent of the patients with low thiamine levels. Differences of this magnitude would occur frequently simply as a result of random sampling and, consequently, lack statistical significance. Without a control group, erroneous inferences could easily be drawn.

TOXICITY OF GLUCOASCORBIC ACID

Restriction of the ascorbic acid intake is known to result in scurvy only in man, the subhuman primates, and the guinea pig. Other animal species have comparable amounts of ascorbic acid in their tissues but are able to maintain these levels by synthesis of adequate ascorbic acid within their own bodies. The site and mechanism for *in vivo* ascorbic acid synthesis in these species are subjects of keen interest to workers in the field of nutrition research.

A compound which would alter the ability of animals to synthesize their own vitamin C would contribute much to the knowledge of the site and mechanism of synthesis. Studies on the administration of an analogue of ascorbic acid, glucoascorbic acid, to rats and mice were designed to determine whether this compound would inhibit synthesis of ascorbic acid (*Nutrition Reviews* **4,** *123* (*1946*)).

D. W. Woolley and L. O. Krampitz (*J. Exp. Med.* **78,** *333* (*1943*)) reported that feeding 10 per cent of glucoascorbic acid as a supplement to an adequate purified diet for a period of ten days to mice and cotton rats caused growth failure, diarrhea, and extensive hemorrhage in the chest, leg, tail, and gingiva. Ascorbic acid administration failed to prevent or cure the lesions.

Other investigators (S. Banerjee and C. A. Elvehjem, *Proc. Soc. Exp. Biol. Med.* **60,** *4* (*1945*)) found that inclusion of a small amount of liver powder into a diet containing glucoascorbic acid entirely prevented the syndrome in rats, chicks, or guinea pigs and allowed the animals to grow well. Ascorbic acid was unable to replace the liver powder for prevention of the lesions.

Banerjee and Elvehjem proposed that the effect of glucoascorbic acid was mediated through depression of the intestinal flora with reduced synthesis of utilizable nutrients which could be supplied in the diet by the liver powder. These investigators also noted that ingestion of large amounts of ascorbic acid resulted in some of the conditions observed with the feeding of similar amounts of glucoascorbic acid.

Somewhat later, B. S. Gould (*Arch. Biochem.* **19,** 1 (*1948*)) reported that there was no antagonism between glucoascorbic acid and ascorbic acid as judged by histologic examination of the jaws of guinea pigs or by estimation of serum phosphatase levels after feeding varying amounts of glucoascorbic acid.

R. J. Gorlin (*J. Dent. Res.* **29,** *208* (*1950*)) studied the effects of adding 5 per cent of glucoascorbic acid to the diet of mice. The control male and female mice gained 9.2 and 8.2 g., respectively, in the two-week experimental period, whereas the experimental males and females lost an average of 1.9 and 2.1 g., respectively. The diarrhea, perianal inflammation, weight losses, and alopecia observed were characteristic of earlier descriptions. However, no subcutaneous hemorrhages or hematomas were observed and the gingivae were not inflamed. Likewise, the microscopic criteria of scurvy were not observed.

Osteoid material was laid down in a normal manner and amount. No increase in the number of osteoblasts was observed in the marrow cavity. There was a profound diminution in the number of proliferating cartilage cells of the epiphyseal plate with a cessation of maturation of the cartilage cells. Calcification of bone and cartilage was reduced in the experimental animals. The abnormalities in the bone and cartilage were believed to be explainable on the basis of inanition.

Histologic examination of the teeth led to the conclusion that the characteristic changes seen in scurvy in the guinea pig were completely absent. There was no evidence of atrophy and disarrangement of the odontoblasts which form the dentin, irregular deposition of dentin, or of hemorrhage into the pulp.

of the prepared food yielded a somewhat lower value, 1.5 mg.

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Banerjee and Elvehjem proposed that the effect of glucoascorbic acid was mediated through depression of the intestinal flora with reduced synthesis of utilizable nutrients which could be supplied in the diet by the liver powder. These investigators also noted that ingestion of large amounts of ascorbic acid resulted in some of the conditions observed with the feeding of similar amounts of glucoascorbic acid.

Somewhat later, B. S. Gould (*Arch. Biochem.* **19,** 1 (*1948*)) reported that there was no antagonism between glucoascorbic acid and ascorbic acid as judged by histologic examination of the jaws of guinea pigs or by estimation of serum phosphatase levels after feeding varying amounts of glucoascorbic acid.

R. J. Gorlin (*J. Dent. Res.* **29,** *208* (*1950*)) studied the effects of adding 5 per cent of glucoascorbic acid to the diet of mice. The control male and female mice gained 9.2 and 8.2 g., respectively, in the two-week experimental period, whereas the experimental males and females lost an average of 1.9 and 2.1 g., respectively. The diarrhea, perianal inflammation, weight losses, and alopecia observed were characteristic of earlier descriptions. However, no subcutaneous hemorrhages or hematomas were observed and the gingivae were not inflamed. Likewise, the microscopic criteria of scurvy were not observed.

Osteoid material was laid down in a normal manner and amount. No increase in the number of osteoblasts was observed in the marrow cavity. There was a profound diminution in the number of proliferating cartilage cells of the epiphyseal plate with a cessation of maturation of the cartilage cells. Calcification of bone and cartilage was reduced in the experimental animals. The abnormalities in the bone and cartilage were believed to be explainable on the basis of inanition.

Histologic examination of the teeth led to the conclusion that the characteristic changes seen in scurvy in the guinea pig were completely absent. There was no evidence of atrophy and disarrangement of the odontoblasts which form the dentin, irregular deposition of dentin, or of hemorrhage into the pulp.

W. G. Shafer (*J. Dent. Res.* **29,** *831 (1950)*) reported the results of experiments with the rat, hamster, and guinea pig, which were designed to study the pathology resulting from administration of glucoascorbic acid. In the rat and hamster experiments, glucoascorbic acid was fed at levels of 5 and 10 per cent of the diet. In no case was there any evidence, grossly or histologically, of a true scorbutic condition.

Aside from the abnormalities attributable to inanition and retarded growth, the only difference between the control and experimental rodents was some congestion in the pulps and periodontal membranes of the latter group. Since the supporting membranes and the newly formed dentin in the incisor are believed to be the most responsive tissues to the deficiency of ascorbic acid, the lack of change in these tissues of the rodents fed glucoascorbic acid indicates the inability of debilitating amounts of this compound to induce a scorbutic condition in these two species of rodents which do not require a dietary source of vitamin C.

In the experiment with guinea pigs, four groups of animals were studied: (1) those fed a scorbutic diet with a supplement of ascorbic acid; (2) those offered the scorbutic diet unsupplemented; (3) those which ingested the scorbutic diet supplemented with both ascorbic acid and glucoascorbic acid; and (4) those fed the scorbutic diet supplemented with glucoascorbic acid. The only group in which frank signs of scurvy were manifest was the one in which the scorbutic diet was fed alone. The guinea pigs in the group fed the scorbutic diet with glucoascorbic acid had appreciably less evidence of scurvy than those fed the unsupplemented scorbutic diet.

From these experimental procedures it becomes evident that administration of glucoascorbic acid does not result in production of scurvy in animals which normally require a dietary source of ascorbic acid nor in those which normally synthesize their own vitamin C. The histologic evidence seems to be indicative of the high degree of inanition to which these animals were subjected secondarily. The route through which the extreme diarrhea and the resulting loss of weight was caused by the glucoascorbic acid is unknown at present. Some similar toxicity is apparently caused by administration of high amounts of ascorbic acid.

The question arises as to whether this condition may be an acidosis, although this probably would not be expected at these levels of fairly high molecular weight compounds. The use of the sodium salt of ascorbic acid or of glucoascorbic acid or the feeding of these free acids with simultaneous, equivalent amounts of a basic compound probably would result in the same toxic manifestations.

URINARY EXCRETION OF VITAMIN B_{12} IN HUMAN BEINGS

Vitamin B_{12} given orally does not seem to be effective in the treatment of pernicious anemia (*Nutrition Reviews* **6,** *245 (1948)*). This is assumed to be the result of impaired absorption from the gastrointestinal tract. On the other hand, when vitamin B_{12} is given orally with normal gastric juice a good therapeutic response may be obtained. To date, the way in which the gastric juice produces this effect with vitamin B_{12} has not been determined. There have been some studies in which gastric juice has been mixed with vitamin B_{12}, but there is no evidence that the normal juice produces any alteration in the vitamin B_{12} or in its microbiologic activity. The gastric juice of human beings is believed to exert its effect by means of enhancing the absorption of vitamin B_{12} from the gastrointestinal tract (*Ibid.* **7,** *146 (1949)*; **8,** *20, 81, 165 (1950)*).

B. F. Chow and co-workers (*Bull. Johns Hopkins Hosp.* **87,** *156 (1950)*) have at-

tempted to use the vitamin B_{12} activity of the urine, estimated by microbiologic assay, as a measure of absorption of the vitamin. Their studies were carried out on ambulatory, normal, young men and some hospitalized patients with typical relapses of pernicious anemia.

Usually a meat-free diet deficient in vitamin B_{12} was provided, but the daily intake of one egg and three glasses of milk was permitted. Vitamin B_{12} was administered either orally or intramuscularly as a solution of the crystalline vitamin. Specimens of urine were assayed for vitamin B_{12} activity by a microbiologic method employing *Lactobacillus leichmannii* as a test organism (H. T. Peeler, H. Yacowitz, and L. C. Norris, *Proc. Soc. Exp. Biol. Med.* **72,** *515 (1949)*).

In an effort to test the ability to determine vitamin B_{12} in normal urine with the above-mentioned method, known amounts of vitamin B_{12} were incorporated in the urine within the range of that present in the specimens of urine. The results demonstrated that recovery was within 20 per cent of the calculated values. Biotin is known to appear in both urine and feces, therefore, in two experiments it was incorporated with a solution of vitamin B_{12} and given either orally or parenterally. It served as a guide to the adequacy of collection. Specimens of urine were collected and assayed for both vitamins.

First, 2 normal persons, after a control period of twenty-four hours were given, intramuscularly, 100 micrograms of crystalline vitamin B_{12}, and a specimen of urine was collected one day before and two days after the injection. No appreciable vitamin B_{12} activity was detectable in the urine prior to administration of the vitamin, but in the first twelve hours after it was injected the activity in the urine was equivalent to the activity of about 50 per cent of the injected material.

The next experiment concerned 3 patients with untreated pernicious anemia. Two of the 3 patients received 100 micrograms of vitamin B_{12} intramuscularly, and the third was given 120 micrograms of vitamin B_{12} plus 10 mg. of biotin. The method used showed that most of the vitamin administered by this means appeared in the urine.

The third experiment was identical with the first except that the 2 normal persons were given 100 micrograms of vitamin B_{12} by mouth, instead of parenterally. However, the urine collected over a period of forty-eight hours after administration of vitamin B_{12} showed no detectable vitamin B_{12} activity.

The fourth experiment involved 6 normal persons who for ten days had meat, poultry, cheese, tomatoes, and seafood excluded from their diet and soybean cake substituted as the main source of protein. Each person, however, was allowed one egg and one quart of milk daily. After a twenty-four-hour control period, 3 persons were given 500 micrograms of vitamin B_{12} intramuscularly and an equal amount of biotin; the remaining 3 persons were given the same amount of the vitamin mixture orally. After six days, 500 micrograms of vitamin B_{12} and of biotin were given orally to 2 persons who had at first received the vitamin mixture parenterally, and to those who had received the mixture orally it was now given intramuscularly.

The bioassay results for vitamin B_{12} showed that this vitamin did not appear in the urine of any of the normal subjects after oral administration of 500 micrograms of vitamin B_{12}. In every case, however, intramuscular injection was followed by prompt appearance of vitamin B_{12} activity in the urine. This activity diminished rapidly about eight hours after injection and the activity in 3 persons had about disappeared in twenty-four hours.

At the end of this study 5 of the 6 persons were given a diet of 400 g. of beef each day and one person received pork and chicken instead of beef, for four days. During these periods of high protein diet, vitamin B_{12} activity in the urine was not appreciable. The

assays on biotin indicated that, unlike vitamin B_{12}, biotin appeared in the urine of all persons to whom it was administered either parenterally or orally. The amount of biotin in the feces was not determined.

In these studies a significant difference in the urinary response of normal persons and of patients with pernicious anemia to the administration of vitamin B_{12} has not been observed. Apparently vitamin B_{12} is excreted rapidly when it enters the body fluids. The authors pointed out that since chemical methods for the identification of vitamin B_{12} were not available they were unable to prove that its activity in the urine of their subjects was brought about by the injections of vitamin B_{12}. Their observations might indicate that vitamin B_{12} is poorly absorbed even by normal persons. It is conceivable also that vitamin B_{12} is altered in some way during its passage through the intestine so that it is no longer possible for the kidneys to excrete it.

One wonders what the results would be in the treatment of pernicious anemia if huge doses, up to several grams of vitamin B_{12}, were given orally without normal gastric juice. It is conceivable that some enzymatic barrier might be overcome, such as occurs with administration of huge doses of vitamin K in cases of prothrombin deficiency due to administration of Dicumarol.

COPPER IN MOTHER'S MILK

Copper has long been classified as a necessary nutrient. It is widely distributed in the adult human body, the total amount found representing 100 to 150 mg. The highest concentrations are found in the liver and the nervous system. Though much is still lacking in the understanding of its role in metabolism, it is known to be implicated in several fundamental biochemical processes.

E. B. Hart, H. Steenbock, J. Waddell, and C. A. Elvehjem (*J. Biol. Chem.* **77,** *797 (1928)*) showed that it is concerned in some way in the synthesis of hemoglobin in the bone marrow. Animals on a milk (low copper) diet fortified with iron developed a hypochromic anemia responding to copper treatment. Similarly, copper deficiency impairs synthesis of catalase. Ascorbic acid oxidase and certain enzymes concerned with pigment formation are copper-containing enzymes. R. H. Follis (*Arch. Path.* **34,** *451 (1942)*) showed that copper deficiency could produce pathologic disturbances of the central nervous system.

Because of these facts, the copper requirement has been considered repeatedly, the needs being considered particularly acute during periods of growth and intense blood formation. The Food and Nutrition Board of the National Research Council recommended in 1942 (*Nutrition Reviews* **1,** *164 (1943)*) that "The requirement for copper for adults is in the neighborhood of 1.0 to 2.0 milligrams a day. Infants and children require approximately 0.05 (mg.) per kilogram of body weight. The requirement for copper is one-tenth of that for iron."

Because cow's milk is extremely low in copper, it has often been felt that in some cases a copper deficiency anemia might develop in infants on an exclusive milk diet, even if they were given some iron (E. Lesné and S. Briskas, *Acta paediat.* **22,** *123 (1938)*). This low copper content and the resulting danger of deficiency can be illustrated by a simple calculation: The average copper content of cow's milk is of the order of 5 to 10 micrograms per cent. The milk consumption of an infant is of the order of 150 ml. per kilogram per day. This in turn would only correspond to 7 to 15 micrograms of copper per kilogram per day, a figure well below the recommended dietary allowance.

If human milk contained as little copper

as cow's milk, or less, it was felt that in at least a few cases, where for any reason the copper requirement of the infant may be increased, it might be advisable to enrich the diet with copper, possibly by administering copper supplements to the mother. A timely study conducted in Denmark by S. Munch-Petersen (*Acta paediat.* **39,** *378 (1950)*) brings much needed information on this interesting question.

Working in the maternity hospital of Aarkus (Jutland) the author first conducted a survey of the copper content of the milk of mothers fed the ordinary hospital diet. The copper content of the milk was observed a great many times on each individual surveyed, samples being taken for analysis both at the beginning and at the end of the nursing periods. The averages found did not differ (54.0 ± 10.9 micrograms per cent at the beginning of nursing periods, 55.0 ± 13.3 micrograms per cent at the end).

The values found were considerably superior to the corresponding figures for cow's milk, in fact closely approaching the optimal allowances. Incidentally, while variations from one individual to another were considerable (the standard deviation has been given above; the range, from the fourth day after delivery, was 20 to 84 micrograms per cent), the level in the same person was, however, fairly constant, the only exception being a pronounced fall on the second and third day after delivery.

Next, the effects of intravenous administration of a copper compound on the copper content of the milk were studied. The compound used was a cuprous salt containing 19 per cent copper. The dose was 100 to 200 mg. Injection was, as could be expected, followed by a considerable rise in serum copper, the increase being of the order of 480 micrograms per cent. Surprisingly, the copper content of the milk was not affected at all, the average copper content of the milk of the subjects submitted to the copper administration being 53.8 ± 12.5 and 54.2 ± 10.5 micrograms per cent, respectively, before and after the administration.

Administered copper does not, therefore, pass into the milk, the secretion of which seems to be regulated as far as copper content is concerned. It is obvious that there can be no rational basis for attempting to supply the child with copper by administering it to the mother, nor should there be normally any need to prescribe any such supplementation for a breast-fed baby.

On the other hand, the presence of a relatively much higher copper content in human milk than in cow's milk, plus the fact that there appears to be a regulatory mechanism insuring the constancy of this high level of copper in human milk, again points to the probable importance of copper in metabolism. It also indicates the possibility that in infants fed artificially, proper supply of copper is one of the factors to be kept in mind.

EGG PRODUCTION AND HATCHABILITY FACTORS

Considerable evidence has been presented that the hatchability of eggs from hens fed a diet low in vitamin B_{12} is markedly decreased and that the hatchability rate is appreciably increased by inclusion of crystalline vitamin B_{12} in such a diet. However, O. Olcese and J. R. Couch (*Poultry Sci.* **29,** *612 (1950)*) found that although the injection of vitamin B_{12} promoted normal hatchability from the sixth through the eighth week, after the ninth week hatchability again decreased.

Similar results were obtained by W. W. Cravens and J. G. Halpin (*J. Nutrition* **37,** *127 (1949)*); *Nutrition Reviews* **8,** *303 (1950)*). Both groups of workers concluded that in

addition to vitamin B_{12} an unidentified factor is required by the hen for egg production and hatchability and that liver fraction "L" and fish solubles are good sources of this factor.

Some reports indicate that the animal protein factor (APF) complex is composed of unidentified factors, in addition to vitamin B_{12}, and that such unidentified factors are required in addition to vitamin B_{12} for chick growth. These findings led J. R. Couch, O. Olcese, B. G. Sanders, and J. V. Halick (*J. Nutrition* **42,** *473 (1950)*) to reinvestigate the requirement of the hen for factors other than vitamin B_{12} for egg production and hatchability.

Single-comb White Leghorn pullets were placed in individual laying cages at approximately 5 months of age. After a pre-experimental period of six weeks, during which time individual egg production and hatchability records were kept, sixteen groups of 4 birds each were selected and fed a diet containing sucrose, soybean protein, soybean oil, fortified fish oil, salts, vitamins, and added methionine and glycine.

Administration of 10 micrograms of vitamin B_{12} orally per hen per week, in addition to the basal diet, resulted in an increase in the percentage of egg production. Oral feeding of the vitamin was less effective than giving 2 micrograms of vitamin B_{12} once a week by injection. Addition of 2.5 g. of an animal protein factor (APF3) containing approximately 70 micrograms of vitamin B_{12} (per kilogram of diet) produced an increase in egg production approximately equal to that of the hens given vitamin B_{12} parenterally. However, feeding of an animal protein factor (APF4) containing 30 micrograms of vitamin B_{12} produced a percentage of egg production which was greater than that of the hens supplemented with vitamin B_{12} or of hens given the animal protein factor which contained a greater quantity of vitamin B_{12}.

Liver fraction "L" and an unspecified type of "liver extract" also increased egg production over that of the hens injected with vitamin B_{12}. It is interesting to note that the percentage of egg production of birds fed dried whey and the active animal protein factor was higher than that of any of the other groups, with the exception of the birds fed the "all-mash" diet.

Hatchability of eggs from hens fed the basal diet decreased decidedly during the first four weeks of the test and few chicks hatched after this time. Addition of dried whey alone failed to improve hatchability appreciably.

Hatchability of eggs from hens injected with crystalline vitamin B_{12} and from hens fed APF concentrates decreased decidedly after the twelfth week of the test, indicating that the hens were depleted of a second factor, over and above vitamin B_{12}, which is required for hatchability after the twelfth week.

Feeding of liver fraction "L" maintained hatchability throughout the test period. This would seem to indicate that this product contains the additional factor necessary for hatchability. The presence of lesser amounts of this factor in other materials was demonstrated by feeding dried whey with APF concentrates and by feeding a high level (5 per cent) of an APF concentrate which was produced as a by-product in the manufacture of aureomycin.

The number of birds used in these experiments was small. However, these data, taken in consideration along with data collected from other types of experiments, indicate that the animal protein factor is not a single entity but rather a complex containing two or more active substances.

HYPERVITAMINOSIS A

In 1944 H. W. Josephs (*Am. J. Dis. Child.* **67,** *33* (*1944*); *Nutrition Reviews* **2,** *148* (*1944*)) described a case of hypervitaminosis A in a child 3 years of age. Since that time additional cases of toxicity associated with excessive ingestion of vitamin A in infants have been described (J. A. Toomey and R. A. Morissette, *Am. J. Dis. Child.* **73,** *473* (*1947*); L. B. Dickey and E. J. Bradley, *Stanford Med. Bull.* **6,** *345* (*1948*); P. E. Rothman and E. E. Leon, *Radiology* **51,** *368* (*1948*); C. T. Fried and M. J. H. Grand, *Am. J. Dis. Child.* **79,** *475* (*1950*); T. C. Wyatt, C. A. Carabello, and M. E. Fletcher, *J. Am. Med. Assn.* **144,** *304* (*1950*)).

J. Caffey (*Pediatrics* **5,** *672* (*1950*)) reported the clinical and roentgenologic findings in 7 patients with hypervitaminosis A, bringing to 15 the number of such cases. Several characteristic features of this syndrome have become apparent. It has been seen only in children between 1 and 3 years of age. There is a long latent period of six to fifteen months of excessive vitamin A intake varying from 75,000 to 500,000 I.U. daily in the form of fish liver oil concentrates.

Onset of the clinical disease is characterized by the simultaneous appearance of hyperirritability, tender swellings over the long bones, and limitation of motion. In some cases pruritis preceded by several weeks or months the onset of the tender swellings. Dry, scaly lips with bleeding fissures at the corners of the mouth; dry, excoriated skin; sparse, coarse hair; and enlargement of the liver have been observed fairly regularly. The swellings were always deep and firmly attached to underlying structures.

The vitamin A content of the blood was elevated above 300 I.U. per 100 ml. in all of the patients on whom this determination was performed. Values as high as 1300 I.U. per 100 ml. were not uncommon. Elevation of the serum alkaline phosphatase occurred regularly. The serum lipids were sometimes elevated and the serum proteins were often depressed. Studies of liver function were within normal limits when performed. Hypoprothrombinemia has not been reported in infants, although it has been observed in hypervitaminotic rats (*Nutrition Reviews* **6,** *261* (*1948*)).

Roentgenograms revealed shell-like hyperostoses (overgrowth of bone) with a zone of diminished density between the subperiosteal thin layer of bone and the external surface of the old cortex. The forearms and the small bones of the feet were most commonly involved, although the lesions on the shanks were often more pronounced clinically. After withdrawal of vitamin A there was symptomatic improvement within a few days, followed by a gradual return to normal of the chemical and roentgenologic abnormalities.

The mechanism by which ingestion of large amounts of fish liver oil concentrates produces this clinical picture is still obscure. This disease in infants has many features in common with hypervitaminosis A in the rat and guinea pig (S. B. Wolbach and O. A. Bessey, *Physiol. Rev.* **22,** *233* (*esp. p. 239*) (*1942*)). It is relatively certain that vitamin A is the causative factor. Acute toxicity in man following the eating of polar bear liver has been attributed to the high vitamin A content of the liver (*Nutrition Reviews* **2,** *63* (*1944*)). Toomey and Morissette (*loc. cit.*) were able to reproduce in their patient the clinical picture of hypervitaminosis A by administration of pure vitamin A.

In the majority of these patients the excessive intake of vitamin A was not known by their pediatricians nor was it recorded in the initial history obtained on admission to the hospital. In 3 cases the history of overdosage was not obtained until two, three, and four years after the patient was first seen. In some instances this history was

elicited only with great difficulty even when the diagnosis had been established. Occasionally a fish liver oil concentrate had been given in the larger doses recommended for cod liver oil. More frequently, enthusiasm of the parents for prophylactic vitamin administration led to excessive intake.

Failure to measure accurately each dose of concentrate was another important cause. One mother reported administration of twenty drops daily, but when it was found that she had been using a 50 ml. bottle each month, it was apparent that her child had been receiving between sixty and seventy drops each day. Some of the older infants drank unknown amounts of oil from the bottle.

It is interesting that to date the use of water-dispersible preparations of vitamin A, which are more rapidly absorbed, has not been associated with these toxic manifestations. This may be due to the lower vitamin A concentration of these products, as suggested by J. M. Lewis, S. Q. Cohlan, and A. Messina (*Pediatrics* **5,** *425* (*1950*)). These workers suggested that the use of bottles of 15 ml. volume for packaging fish liver oil concentrates would reduce the possibility of ingestion of excessive quantities.

Greater caution should be exercised by public health nurses, physicians, and others who instruct mothers concerning infant feeding, so that misunderstandings as to dosage and form of vitamin A supplements do not arise, and so that overenthusiasm for vitamin administration on the part of those caring for children does not lead to detrimental results.

ATURE OF IODINE COMPOUNDS IN BLOOD AND URINE

The nature of organic iodine compounds in blood and urine of human subjects, both normal and those with hyperthyroidism, has long been a matter of dispute. Certain investigators have presented evidence that free thyroxine is present in the circulating blood in an amount sufficient to account for an appreciable portion of the blood iodine.

A. Taurog and I. L. Chaikoff (*J. Biol. Chem.* **176,** *639* (*1948*)) concluded that thyroxine is present in circulating blood in the form of a loosely bound protein complex and that this loosely bound protein-thyroxine complex accounts for about 90 per cent of the organic iodine in blood serum.

W. T. Salter and M. W. Johnston (*J. Clin. Endocrinol.* **8,** *911* (*1948*)) claimed that thyroxine added to blood serum did not behave in the same way as did the naturally-occurring organic iodine of serum.

For a long time it has been known that after administration of large amounts of diiodotyrosine to animals, this compound is excreted in the urine. However, in normal human subjects it has been both claimed and denied that organic iodine compounds are excreted in the urine.

Several papers have appeared which demonstrate the chemical nature of at least some organic iodine compounds in blood and urine.

J. E. Rall (*J. Clin. Endocrinol.* **10,** *996* (*1950*)) administered radioiodine to 8 patients with hyperthyroidism, 5 with euthyroidism, and 3 with hypothyroidism, and by chemical procedures separated thyroxinelike, diiodotyrosinelike, and iodine fractions and determined the radioiodine content of these fractions in both blood and urine.

In the majority of the patients with euthyroidism and those with hyperthyroidism most of the radioactivity was in the thyroxinelike fraction. The greater the time interval between administration of radioiodine and separation of the fractions, the more radioactivity there was in the thyroxinelike fraction.

In the urine of patients with hyperthyroidism, from 12 to 40 per cent of the

urinary radioiodine was present in organic form by the third day following administration of radioiodine. In the patients with euthyroidism less than 2 per cent of the total urinary radioiodine was in the form of organic iodine compounds.

Studies on the organic iodine compounds by means of butanol, alkali, and silver solubilities demonstrated the presence of thyroxinelike and diiodotyrosinelike fractions in the urine of both patients with euthyroidism and those with hyperthyroidism. That thyroxine was present as part of the organic urinary iodide was also suggested by recrystallization technics and filter paper chromatography.

Three other groups of workers (A. Taurog, I. L. Chaikoff, and W. Tong, *J. Biol. Chem.* **184,** *99* (*1950*); J. C. Laidlaw, *Nature* **164,** *927* (*1949*); and J. Gross, C. P. Leblond, A. E. Franklin, and J. H. Quastel, *Science* **111,** *605* (*1950*)), by the use of radioactive iodine and the technic of paper partition chromatography, have demonstrated that the major organic iodine compound in rat plasma is thyroxine. In addition, Gross *et al.* (*loc. cit.*) found that when whole plasma was analyzed by radioautography of two-dimensional buffer chromatograms, the location of the radioactive material corresponded to that of the plasma proteins. These workers concluded that thyroxine, after its release by the thyroid gland, circulates in combination with plasma.

THE BIOCHEMICAL DEFECT IN PERNICIOUS ANEMIA

In view of the many recent reports concerning the metabolic functions of pteroylglutamic acid and vitamin B_{12} and the interrelationship of these vitamins and purine and pyrimidine metabolism in bacterial systems (*Nutrition Reviews* **8,** *20*, *260* (*1950*)), it is not surprising that there should be considerable speculation concerning the biochemical defect in pernicious anemia. This speculation has been enhanced by the discoveries that pteroylglutamic acid is a potent hematopoietic agent in pernicious anemia and related macrocytic anemia in doses measured in milligrams, that thymine (5 methyl uracil) can replace folacin if administered to patients with pernicious anemia in doses of 12 to 15 g. daily, and that vitamin B_{12} is active in these conditions in microgram quantities.

R. W. Vilter *et al.* (*Blood* **5,** *695* (*1950*)) have taken the opportunity of testing in patients with various types of megaloblastic anemia some of the possibilities implied in these relationships. They conclude that their results support the proposition that folacin and at least one other catalyst facilitate the formation of thymine and other essential purines and pyrimidines from intermediary compounds such as uracil; and that vitamin B_{12} acts at a later stage of a series of reactions which terminate in the synthesis of nucleoprotein.

Forty-two patients with pernicious anemia were placed on a regimen of 30 mg. of folacin three times weekly given orally for many months. Follow-up observations were obtained in 36 of these patients. Of these 36 persons, 11 were maintained in satisfactory hematologic and neurologic remission on this dosage; 4 expired of diseases unrelated to their anemia but were maintained satisfactorily up to their death; 5 experienced hematologic relapse; 7 suffered neurologic relapse; 7 showed manifestations of both hematologic and neurologic relapse concomitantly; and two developed manifestations of hematologic relapse followed shortly by neurologic relapse. The hematologic relapses (14 patients) occurred on the average of thirty months after the therapy was changed from liver extract to folacin. Erythrocyte counts and hemoglobin levels in these patients fell to values as low as 1,500,000 cells per cu. mm. and 7.5 g. per 100 ml.,

respectively. Macrocytosis appeared and white blood counts tended to fall. However, typical megaloblastic bone marrow was not present.

Patients who had severe neurologic relapse as well as hematologic relapse were treated with refined liver extract or vitamin B_{12} and had satisfactory neurologic and hematologic recovery.

Six patients who had definite signs of hematologic relapse with little or no neurologic deterioration were given various therapeutic tests. Two received thymine, 15 g. daily for ten days. In one of these, reticulocytosis occurred (10 per cent) and the erythrocytes and hemoglobin rose from 1,780,000 cells per cu. mm. and 8.9 g. per 100 ml. to 2,950,000 cells per cu. mm. and 11.0 g. per 100 ml., respectively. Subsequent treatment with refined liver extract did not induce further reticulocytosis and the erythrocytes rose slowly. Signs of neurologic degeneration cleared rapidly. The other patient who received thymine had a rise in reticulocytes (5 per cent) but the erythrocytes remained between 2,800,000 and 3,000,000 cells per cu. mm.

Four patients were given increased doses of folacin (50 mg. daily). Within four weeks after this change in therapy was instituted, the peripheral blood counts rose significantly but macrocytosis persisted. After two to eleven months on the increased dose of folacin the values fell again, this time with the appearance of combined system disease in all patients. Examination of the bone marrow showed mild or moderate evidence of erythrocyte maturation deficiency but typical megaloblastic arrest was not a prominent feature. Two of the 4 patients were then treated with vitamin B_{12} parenterally. Neurologic improvement was rapid in each instance but the hematologic response was slow.

Three patients with pernicious anemia, not previously treated with folacin, were given 15 to 30 g. of uracil by mouth daily for ten days. The first subject had a reticulocyte response to 6.8 per cent on the ninth day. Bone marrow taken at this time showed a shift from a preponderance of megaloblasts to normoblasts. The second patient treated with uracil had no reticulocyte response or erythrocyte rise and no change in the bone marrow on the tenth day. The third patient had a reticulocyte rise from 6 per cent to 15 per cent on the ninth day. During a subsequent ten-day period of uracil administration there was a second reticulocyte response to 7 per cent on the eighth day. The bone marrow progressively reverted to a normoblastic type during these two periods and the erythrocytes and hemoglobin rose from 1,400,000 to 2,840,000 cells per cu. mm. and from 6.3 g. per 100 ml. to 11.6 g., respectively. The mean corpuscular volume, however, remained elevated.

A 33-year-old female with pernicious anemia of pregnancy and nutritional cirrhosis was observed during the administration of successive courses of uracil (30 g. orally per day for ten days), methionine and choline (6 g. and 3 g., respectively, per day for ten days), and thymine (15 g. orally per day for ten days). There was no reticulocytosis, erythrocyte rise, or change in the megaloblastic bone marrow after uracil administration. Following methionine and choline the reticulocytes increased to 7.4 per cent on the ninth day and the erythrocytes increased 800,000 cells per cu. mm. The bone marrow changed from megaloblastic to normoblastic. Following administration of thymine, a second reticulocytosis occurred, the erythrocytes rose, and the bone marrow reverted to normal.

The above observations along with observations in bacterial metabolism have led the authors to postulate the existence of a chain type of reaction. According to this scheme, folacin together with unknown substances activates the formation of purine and pyrimidines from amino acids and probably facilitates the interconversion of these nitrogenous bases. An interconversion reac-

tion of this type is illustrated by the methylation of uracil to form thymine.

Vitamin B_{12} on the other hand probably activates the formation of nucleosides from the purines and pyrimidines. According to this hypothesis folacin induces hematologic remissions in patients with pernicious anemia by a "mass action" effect. It appears, however, that each increase in the dose of folacin, because it induces a remission by forcing the reaction to completion, tends to deplete the body of vitamin B_{12}. If this is true, then probably the depletion of the scant supply of vitamin B_{12} available to patients with pernicious anemia may be the reason why every patient developed neurologic relapse as the dose of folacin was increased in an attempt to combat the hematologic relapse.

The authors offer two explanations for the observation that thymine induced a response in patients with pernicious anemia even after folacin had lost its effect. Either thymine acts through a parallel reaction and circumvents the need for folacin and the enzyme systems related to it, or thymine is the product of a reaction activated by folacin and unknown substances.

The authors admit that these speculations are highly theoretical, only partially proved, and replete with assumptions. They are, however, interesting and there is evidence, now rapidly accumulating, that both folacin and vitamin B_{12} are concerned in the synthesis or utilization of one-carbon fragments (*Nutrition Reviews* **8,** *145, 301 (1950)*). This might help to explain the therapeutic effectiveness of methionine and choline in the patient with macrocytic anemia of pregnancy.

FECAL NITROGEN AND FAT IN LIVER DISEASE

The relationship between caloric intake and nitrogen balance has been repeatedly demonstrated in studies of man. Thus nitrogen balance may be influenced by variations in the dietary intake and in the absorption of fats and carbohydrates which are the major sources of calories. In the study of therapeutic measures in liver disease, nitrogen balance has been used as a measure of the efficacy of certain nutrient factors (*Nutrition Reviews* **8,** *238, 284 (1950)*). The problem of fat and nitrogen loss and thus calorie loss in the feces of patients with hepatic disease has been investigated by J. B. Gross, M. W. Comfort, E. E. Wollaeger, and M. H. Power (*Gastroenterology* **16,** *140 (1950)*). This report also contains a summary of previous work on the effects of liver disease on fecal excretion of fat and nitrogen.

Fecal excretion of solids, fat, and nitrogen was studied in 10 patients with liver disease who were receiving a constant 2463 calorie diet containing 101.6 g. of fat and 117 g. of protein. Eight of the patients were suffering from hepatitis (3 serum, 4 infectious, and one chronic in type) of varying degrees of severity and duration, and 2 of them had Laennec's cirrhosis, one of whom had ascites. All had hyperbilirubinemia, and fecal urobilinogen was within normal range or above in all except the subject with chronic hepatitis. In this patient urobilinogen was present but in reduced amounts.

Total fecal lipids in the cases of hepatitis were found to be elevated in 7 of the 8 patients and at the upper limit of normal in one. Comparisons were based upon a previous study of fecal fats in a group of normal human beings receiving the same diet (E. E. Wollaeger, M. W. Comfort, and A. E. Osterberg, *Gastroenterology* **9,** *272 (1947)*). In the 2 subjects with cirrhosis, fecal fats were normal, although the literature reviewed by the authors indicates that 17 of 22 such cases reported showed increased fecal fats over the normal average figure of 10 per cent of the fat ingested.

Subsequent studies of 2 patients within

this hepatitis series revealed that fecal fat excretion fell toward normal values with clinical improvement and clearing of the jaundice. An attempt to lower fecal fat by administration of desiccated hog bile to the patient with chronic hepatitis resulted in diarrhea and no reduction in fat loss.

Average fecal solids were not significantly increased over those for normal persons but there was a direct correlation between fat content and solids. Fecal nitrogen excretion was also not increased above normal in either the patients with hepatitis or those with cirrhosis. Average daily calorie loss based upon fecal fat and nitrogen was, however, nearly twice that for normal persons (153.8 versus 78.7) and was highest (105 to 308) in patients with the greatest fecal fat losses.

The authors discuss the possible m chanisms responsible for the steatorrhea found in the patients with liver disease and ascribe it to the possibilities of portal hypertension, external pancreatic insufficiency, or alteration in the secretion of bile. Portal hypertension is eliminated as a cause in view of the variable relationship between its presence or absence and the degree of steatorrhea.

In connection with the second possibility, a study of external pancreatic function in hepatic disease is also reported (J. B. Gross, M. W. Comfort, E. E. Wollaeger, and M. H. Power, *Gastroenterology* **16,** *151* (*1950*)). Duodenal contents were analyzed for volume, bicarbonate concentration, and total amylase, trypsin, and lipase before and after secretin administration. Data were obtained on 10 patients with hepatic disease, 8 of whom were included in the study of fecal fat and nitrogen (*loc. cit.*). The results of this study revealed no remarkable differences in enzyme elaboration between normal individuals and patients with liver disease. Bicarbonate concentration, however, which normally rises after secretin administration, was low in 5 of 9 patients so studied. Total bicarbonate content in these subjects nevertheless was normal or above due to increased volumes of fluid secreted.

In view of these findings the investigators suggest that the impaired formation and secretion of bile salts by a damaged liver is the best explanation of steatorrhea. In their opinion, simple quantitative reduction in the amount of bile released into the intestine is eliminated by the findings of normal or greater amounts of fecal urobilinogen. No evidence for diminished bile salt production in liver disease is presented, however, and further studies of the effect of these salts in such patients appear to be indicated if such can be accomplished without the production of diarrhea.

The authors indicate that rarely is loss of calories in the feces a therapeutic problem in cases of liver disease. It should perhaps be pointed out, however, that nitrogen balance studies in individual patients may be altered if fecal fat and calorie loss are markedly increased. The effect of small reductions or increments in calories in patients with liver disease has not been clearly defined but certain studies (J. M. Strang, H. B. McClugage, and F. A. Evans, *Am. J. Med. Sci.* **181,** *336* (*1931*)) indicate that variations of 50 to 100 calories of carbohydrate in low calorie diets may prevent or produce nitrogen wastage in obese persons.

In view of the caloric loss in the feces in patients with liver disease, nitrogen balance changes during administration of lipotropic agents or other nutrient factors to patients with hepatitis or cirrhosis should be interpreted cautiously. Other aspects of nitrogen balance in such subjects have been discussed previously (*Nutrition Reviews* **6,** *298* (*1948*); **8,** *284* (*1950*)).

VITAMIN E IN HUMAN LIVER DISEASE

Animals receiving diets low in the sulphur-containing amino acids may develop massive necrosis of the liver which can be partially prevented by the administration of tocopherol (*Nutrition Reviews* **7,** *302 (1949)*; **8,** *116, 204, 310 (1950)*). Moreover, vitamin E may protect animals against liver damage following carbon tetrachloride poisoning (E. L. Hove, *Arch. Biochem.* **17,** *467 (1948)*). With this suggestive evidence in mind, G. Klatskin and W. A. Krehl (*J. Clin. Invest.* **29,** *1528 (1950)*) undertook a study of plasma tocopherol levels and tocopherol tolerance tests in patients with liver disease.

Twenty-three normal, healthy adults and 57 hospitalized patients without evidence of liver disease, were compared with 43 patients with liver disease, most of them suffering from Laennec's cirrhosis. The diets of the normal adults were not controlled. The hospitalized non-liver disease patients were provided with a routine hospital diet furnishing 1 g. of protein and 30 calories per kilogram of body weight per day without supplementation. The mean plasma tocopherol levels of these three groups of patients were 1.23, 1.02, and 0.95 mg. per cent respectively. The only groups considered to be significantly different were the first and the third, that is, the normal controls and the patients with liver disease.

The authors sought factors which might have influenced the plasma tocopherol level, but found that it did not correlate with liver function tests, presence or degree of jaundice, age, or serum cholesterol concentration. Neither was there a clearcut relationship between changes in hepatic function and the plasma tocopherol level.

The oral administration of 500 mg. of fat soluble DL-alpha tocopherol acetate and of the water soluble D-alpha tocopheryl disodium phosphate resulted in a significantly slower rise in the blood tocopherol curve and a delayed peak in the patients with liver disease. Attempts to study tolerance curves following intramuscular or intravenous injections of tocopherol preparations were unsuccessful. For this reason the delayed rise and low peak of the tocopherol tolerance curve in liver disease could not be blamed definitely upon impaired absorption. Further, the fact that the patients with chronic liver disease had been consuming a diet presumably sub-optimal with regard to tocopherol suggested that antecedent diets may have played an important role in the finding of low tocopherol levels in both the patients with liver disease and the hospitalized patients without evidence of hepatic disturbance.

The authors concluded that although a disturbance in tocopherol absorption or intermediary metabolism appeared evident in their patients with liver disease, no conclusion as to its etiology could be formulated.

ENVIRONMENT AND VITAMIN CONTENT OF FOOD PLANTS

An important variable in the climatic complex is temperature. The effect of this factor has been studied in relation to content of ascorbic acid, but other vitamins have been essentially neglected. Even the results on ascorbic acid are somewhat contradictory. Consideration of such a factor as temperature is very important in relation to surveys made of plant varieties and strains preliminary to plant breeding programs designed to improve the vitamin content of plant parts used for human or animal food. Neglect of such a factor, if it is significant, could well nullify the work of years of effort in

plant breeding. Thus a recent contribution by F. G. Gustafson (*Plant Physiol.* **25,** *150 (1950)*) is particularly welcome.

In studying the influence of temperature on the vitamin content of green plants, Gustafson employed 10 species, ranging in age from 2 weeks to 7 months. This work was restricted to stems and leaves, which in some species are not important economically and may or may not reflect what might be found in the fruit of the plant. Only certain vitamins of the B complex were studied, namely thiamine, riboflavin and nicotinic acid. Two temperature ranges were employed in the greenhouse, 10 to 15° and 28 to 30° C. Prior to analysis, the plants were kept at these temperatures at least a week and usually for several weeks or months.

Not all plants responded to the same extent to temperature in their synthesis of vitamins nor were all vitamins affected in the same way. Plants which make their best growth during the warm part of the summer had a higher vitamin content at the higher temperatures. This was true of tomatoes, beans, and soybeans. Likewise, plants like cabbage, broccoli and spinach, that grow better during the cooler part of the summer and in spring and fall, tended to have a higher vitamin content at the lower temperature. Clover, lupine, peas, and wheat were intermediate between the above two groups. In the latter group, the lower temperature favored a higher vitamin content in general but there were exceptions. Riboflavin was consistently higher at the lower temperature in the plants of this group.

The differences reported here were as great as 61 per cent and often 15 to 30 per cent. Whether these differences arise in the first group from increased rate of synthesis of vitamins at higher temperature relative to their rate of utilization, or whether the vitamins are lost more slowly at lower temperatures in the case of plants in the second group, is not known. To the practical individual, knowledge of the optimum temperature for vitamin content should be useful, for he can make wider use of those plants in his diet during seasons when their high vitamin content is favored. The plant breeder should have a knowledge of these relationships for his particular species as a preliminary background for genetic work designed to change the composition of a strain or variety.

An extensive statistical treatment has been given to a large body of data on the vitamin contents of wheat, corn, and oats grown in three types of soil fertility experiments by C. H. Hunt, L. D. Rodriguez, and R. M. Bethke (*Cereal Chem.* **27,** *79 (1950)*). After a five-year rotation fertility experiment they conclude that season affected the thiamine and riboflavin contents of wheat, corn, and oats, the niacin content of corn and oats, and the pantothenic acid content of corn. The effect of fertilizers (other than lime) on the vitamin content of wheat was selective. Phosphorus alone increased the thiamine content, nitrates increased the niacin, while nitrates and potash in combination increased the pantothenic acid content of wheat. Potash alone decreased thiamine of wheat. Nitrates increased the thiamine content of oats. In corn, potash decreased the thiamine content, as did a dry season compared to a normal season. Wheat grown in a normal year had more riboflavin than that grown in a wet year. The reverse was true for oats. So many fertility and soil factors were varied in this work that definite conclusions are difficult to make.

NOTES

Absorption of Oily and Water-Miscible Vitamin A in Steatorrhea

The recent interest in and availability of dispersing agents have stimulated the investigation of absorption not only of fat but of fat-soluble substances, especially vitamin A. There is clearcut evidence that administration of vitamin A in a water-miscible form produces a more rapid increase in the blood concentration and higher blood levels in normal individuals and in patients with steatorrhea than occurs following administration of the oily form of the vitamin. Fecal excretion, at least in normal infants, is markedly less with the aqueous than with the oily preparation (*Nutrition Reviews* **6,** *248 (1948)*; **7,** *205 (1949)*).

B. C. Barnes, E. E. Wollaeger, and H. L. Mason (*J. Clin. Invest.* **29,** *982 (1950)*) have confirmed the higher plasma curves and lower fecal losses of vitamin A following administration of an aqueous dispersion compared with the oily ones. Five patients with nontropical sprue and steatorrhea were also studied and an additional patient with diabetes mellitus and associated steatorrhea was included in the series. Though there was an excessive excretion of vitamin A in the feces of the patients with nontropical sprue following administration of both preparations, there was better absorption of the water-miscible preparation, as indicated by diminished fecal excretion as well as higher plasma curves. However, the patient with diabetes mellitus had large fecal losses with both preparations without a significant difference between them. These almost identical losses occurred despite a normal plasma vitamin A curve following oral ingestion of the water-miscible preparation and a flat curve following the oily material.

The importance of measuring fecal losses of vitamin A is pointed out by the authors, since only in this way can the percentage of the administered dose absorbed be determined. From these results it would appear that blood curves following oral administration do not necessarily reflect accurately the absorption of the vitamin.

Nutritive Value of Milk and Milk Products

Some reviews prepared by the National Institute for Research in Dairying, University of Reading, England, are of interest to nutritionists. The review by S. K. Kon and K. M. Henry (*J. Dairy Res.* **16,** *68 (1949)*) dealing with the nutritive value of milk and milk products makes reference to nine hundred and thirteen published papers. The subject of dairy chemistry and physics, reviewed by R. Aschaffenburg and J. A. F. Rook (*Ibid.* **16,** *390 (1949)*), covers a literature survey of seven hundred and fifty-two published papers. These reviews in general cover the period 1942 to 1947 and refer to a considerable volume of literature that was largely inaccessible during the war years.

Ascorbic Acid in Walnut Hulls

The immature walnut, or its green hull, is one of the richest natural sources of ascorbic acid. It has been reported to contain from 2 to 2.5 per cent of ascorbic acid on a wet basis (16 to 20 per cent on a dry weight basis).

Workers at the Western Regional Research Laboratory in Albany, California, (A. A. Klose *et al.*, *Ind. Eng. Chem.* **42,** *387 (1950)*) have developed a chemical process for recovery of ascorbic acid from waste green walnut hulls. The procedure consists of extraction of the hulls with dilute sulfur dioxide solution. The extract is purified by adsorption on, and elution from, anion exchange resin, and the ascorbic acid is crystallized from the concentrated decolorized eluates with yields of 25 to 50 per cent.

The California workers point out that the procedure cannot be considered an economic commercial source of ascorbic acid unless uses can be found for the equipment during off-seasons. At least 50,000 tons of hulls are produced yearly in California alone, but

they are available for extraction only one to two months of the year.

Vitamins in Cereals and Grasses

Since 85 per cent of the oat crop is utilized in livestock feed, the composition of this crop in relation to animal nutrition is important, chiefly with regard to certain members of the vitamin B group, thiamine, niacin, riboflavin, and pantothenic acid. As with many other crop plants, most of the effort in development of new varieties has emphasized disease resistance, yield, and other desirable agronomic characteristics. Methods for comparisons of vitamin contents have been largely neglected and of course many of them were not available until relatively recently. In the literature, contents of oats have been reported as follows: thiamine 6 to 10.3, niacin 4.9 to 7.0, and riboflavin 1.21 to 1.35 micrograms per gram.

K. J. Frey and G. I. Watson (*Agronomy J.* **42,** *434* (*1950*)) recently reported on contents of certain vitamins in 16 varieties of oats, all grown under similar environmental conditions. Niacin content ranged from 4.4 to 11.7 micrograms per gram, while thiamine content ranged from 5.37 to 9.69 micrograms per gram. All varieties contained enough thiamine to meet dietary requirements of swine as set up by the National Research Council. Riboflavin content ranged from 1.05 to 1.87 and pantothenic acid content ranged from 6.3 to 12.7 micrograms per gram. All varieties were too low in riboflavin, and all but one too low in pantothenic acid, for swine. Significant positive correlations were found between the contents of protein and both niacin and thiamine, and between niacin and both riboflavin and thiamine. Pantothenic acid content was negatively correlated with protein and all 3 vitamins. The ranges presented above would indicate good opportunities for improvement of oats, nutritionally speaking, by the plant breeder.

J. W. Hibbs and M. W. Evans (*Agronomy J.* **42,** *94* (*1950*)) analyzed 3 varieties of timothy at different stages of development. As the season advanced and per cent dry matter increased, the carotene content decreased. Thus, later varieties contained more carotene than earlier ones harvested on the same date. For hay, it is thus best to cut timothy not later than the early bloom stage. Such a time would also take advantage of higher levels of thiamine, riboflavin, and protein before excessive amounts of cellulose and lignin develop as fiber content.

Dealing only with younger cereal grasses, F. Moyer and co-workers (*Agronomy J.* **41,** *344* (*1949*)) analyzed barley, oats, rye, wheat and ryegrass, clipped at 3 to 4 inches during 3 seasons in both fall and spring. Powders made from such material, carefully dried, have been used considerably as supplements to both poultry feed and human food specialties. They were high in carotene, and compared favorably in content of thiamine, riboflavin, and nicotinic acid with such common vitamin supplements as alfalfa leaf meal, dried buttermilk, dried whey, and other high protein carriers. No differences were found among the cereals, but differences due to sampling date were significant for all vitamins. Drying these clippings at 65°C. resulted in a loss of riboflavin, no change in nicotinic acid, and an apparent increase in pantothenic acid.

These results again serve to emphasize that the requirements for minerals by animals cannot be stated dogmatically without reference to the composition of the diet. Particular attention must be paid to the concentration of all elements in the diet where the requirements for any given element are considered.